

Mesomeric forms of *p*-benzoquinone. (a) anionic; (b),(c) neutral; (d) cationic.

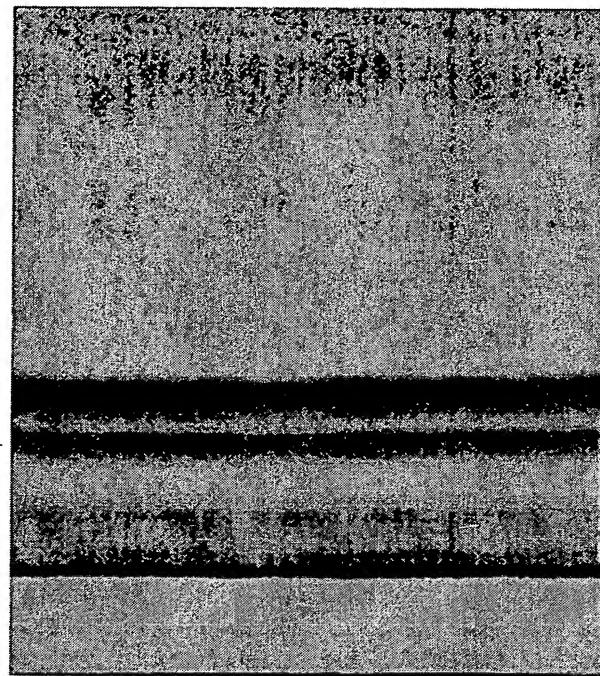


Fig. 2

*Band thin layer chromatography of the methanol solution after lyophilization
(step 5) —→ Indicates the band of the cs-oxidant.*

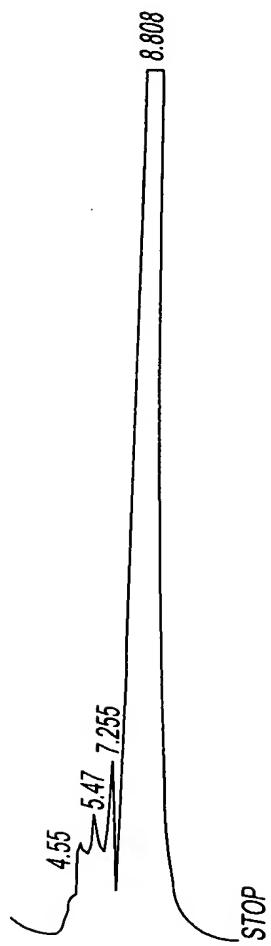
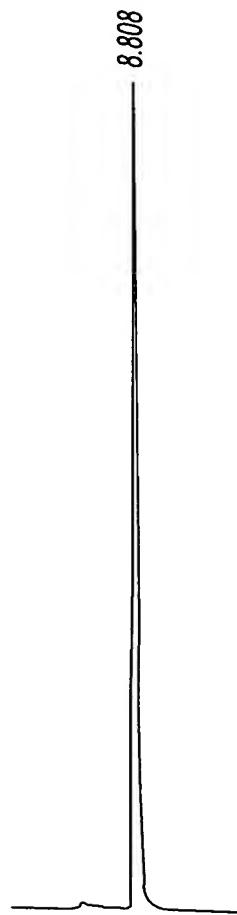


Fig. 3

HPLC profile the butanol extract after TLC. The cs-oxidant (step 6) eluted as a major peak at the retention time of 8.808 min. The amount of cs-oxidant eluted was $\approx 12 \mu\text{g}$.



| | | | | | |
|-------------|-------|--------|----|------|------|
| CHROMATOPAC | C-R6A | | | | |
| SAMPLE NO | 0 | FILE | 0 | | |
| REPORT NO | 35 | METHOD | 41 | | |
| PKNO | TIME | AREA | MK | IDNO | NAME |
| 1 | 8.808 | 387815 | | | |
| TOTAL | | 387815 | | 100 | |

Fig. 4

HPLC profile of the pure cs-oxidant, eluted at the retention time of 8.808 min.

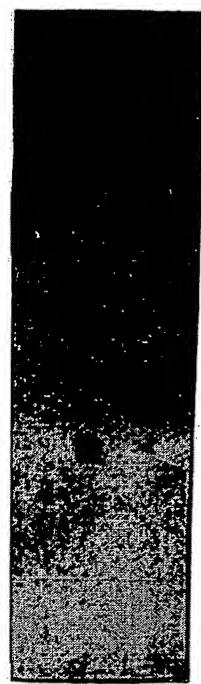
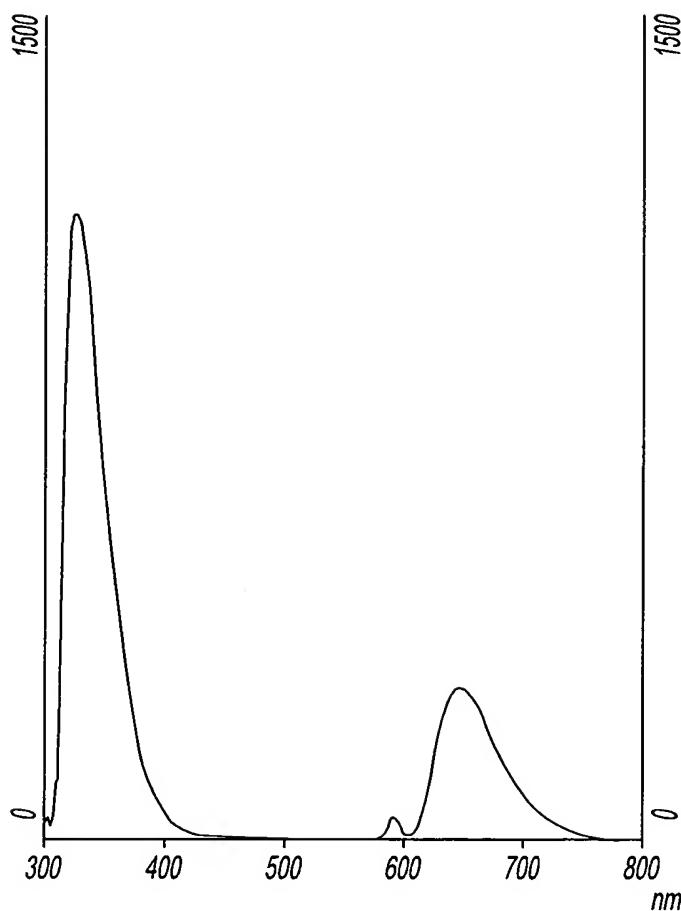


Fig. 5

Thin layer chromatography of the pure cs-oxidant ($R_f = 0.26$)



CX WAVELENGTH 293nm

CX BANDPASS 5 nm

CM BANDPASS 5 nm

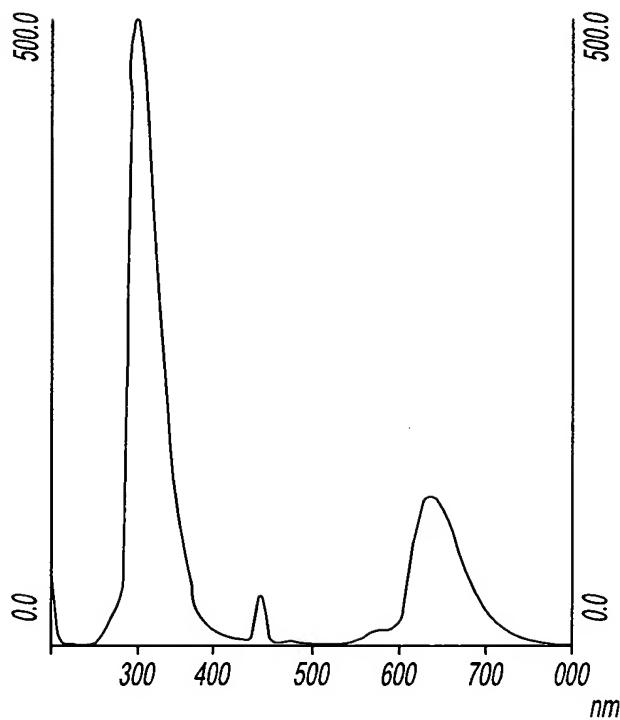
SCAN SPEED: 240.0 nm/min

RESPONSE 2 sec

| NO. | PEAK | VALLEY |
|-----|----------------|----------------|
| 1 | 329.6 nm 1150 | 299.0 nm 20.04 |
| 2 | 591.4 nm 44.52 | 560.2 nm 0.764 |
| 3 | 651.4 nm 201.7 | 603.0 nm 7.563 |

Fig. 6a

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The excitation was at 293 nm and the emission scanning was measured from 300 nm to 800 nm. The emission maxima were at 329.6 nm and at 651.4 nm.

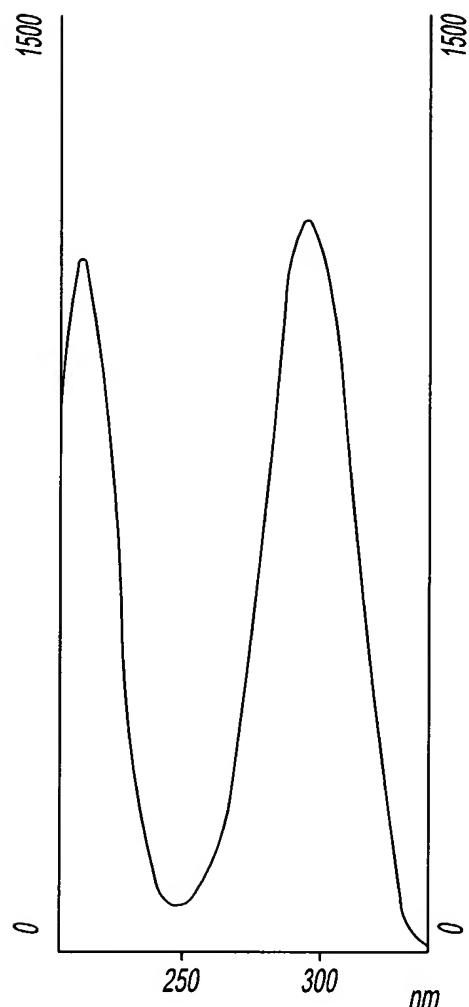


| | | | |
|---------------|--------|-------------|------------|
| CX WAVELENGTH | 224 nm | SCAN SPEED: | 240 nm/min |
| CX BANDPASS | 5 nm | RESPONSE | 2 sec |
| CM BANDPASS | 5 nm | | |

| NO. | PEAK | | VALLEY | |
|-----|----------|-----------|----------|-----------|
| | nm | Intensity | nm | Intensity |
| 1 | 329.6 nm | 502.2 | 261.2 nm | 0.524 |
| 2 | 454.6 nm | 41.39 | 228.6 nm | 3.647 |
| 3 | 405.4 nm | 3.563 | 476.4 nm | 2.356 |
| 4 | 652.6 nm | 121.2 | 527.6 nm | 1.114 |

Fig. 6b

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The excitation was at 224 nm and the emission scanning was measured from 225 nm to 800 nm. The emission maxima were at 329.6 nm and at 652.6 nm.



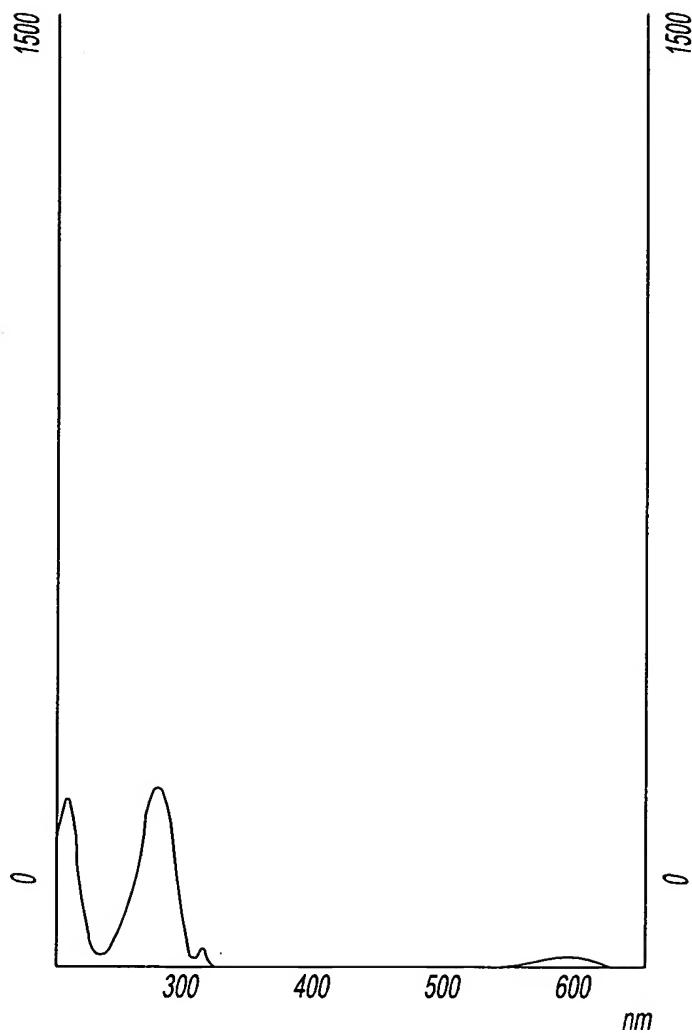
EX BANDPASS 5 nm
EM BANDPASS 5 nm

EM WAVELENGTH 330 nm
SCAN SPEED: 240 nm/min
RESPONSE 2 sec

| NO. | PEAK | VALLEY |
|-----|----------|-------------------------------|
| 1 | 228.2 nm | 1115 252.4 nm 77.46 |
| 2 | 293.8 nm | 1174 |

Fig. 7a

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The emission was at 330 nm and the excitation scanning was measured from 220 nm to 325 nm. The excitation maxima were at 228.2 nm and at 293.8 nm.



| CX BANDPASS | 5 nm | EM WAVELENGTH | 651 nm |
|-------------|----------|---------------|----------------|
| CM BANDPASS | 5 nm | SCAN SPEED: | 240 nm/min |
| | | RESPONSE | 2 sec |
| | | | |
| NO. | PEAK | VALLEY | |
| 1 | 229.2 nm | 267.2 | 252.2 nm 19.04 |
| 2 | 294.8 nm | 200.0 | 320.0 nm 7.691 |
| 3 | 325.0 nm | 31.90 | 370.2 nm 0.095 |
| 4 | 597.0 nm | 21.70 | 642.0 nm 0.513 |

Fig. 7b

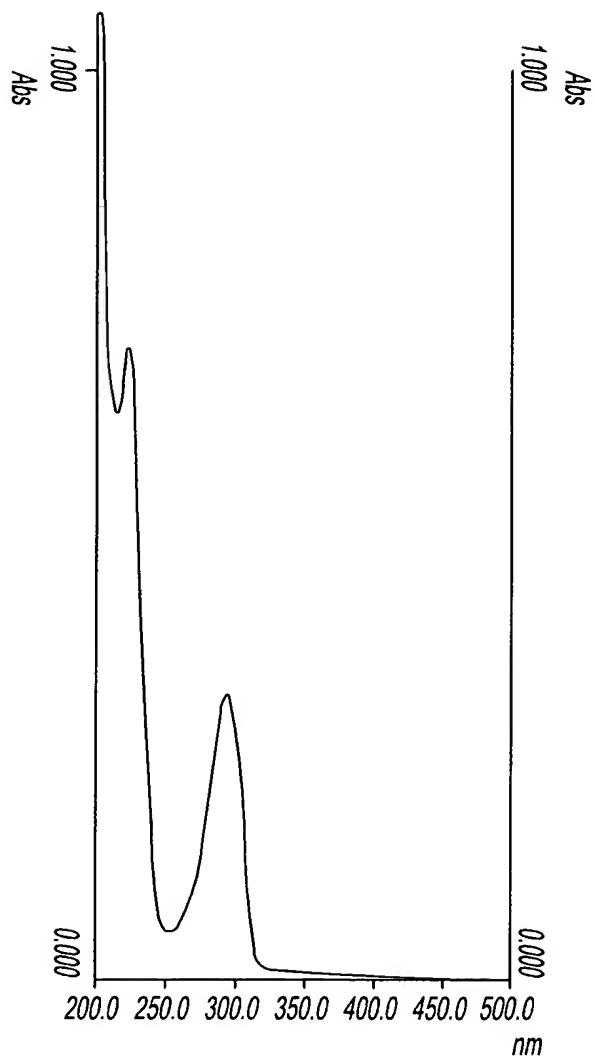
Fluorescence spectroscopic profile of the cs-oxidant in methanol. The emission was at 651 nm and the excitation scanning was measured from 220 nm to 650 nm. The excitation maxima were at 229.2 nm and at 294.8 nm.

10/35



Fig. 8

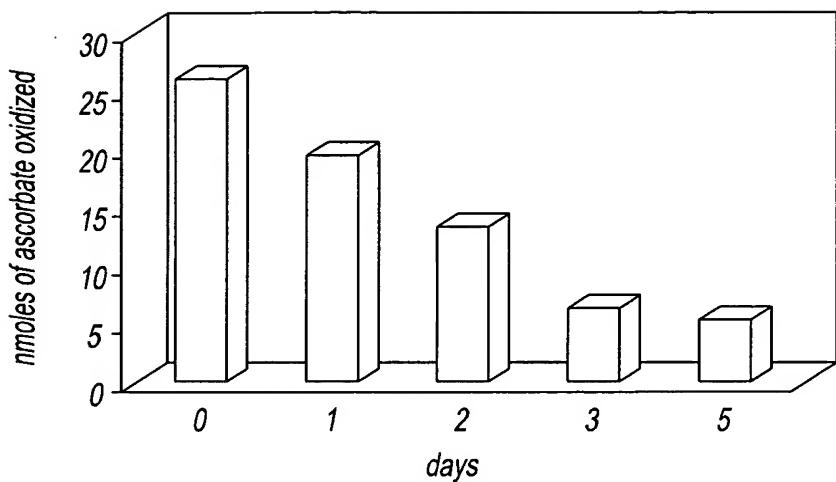
Crystal structure of the pure cs-oxidant



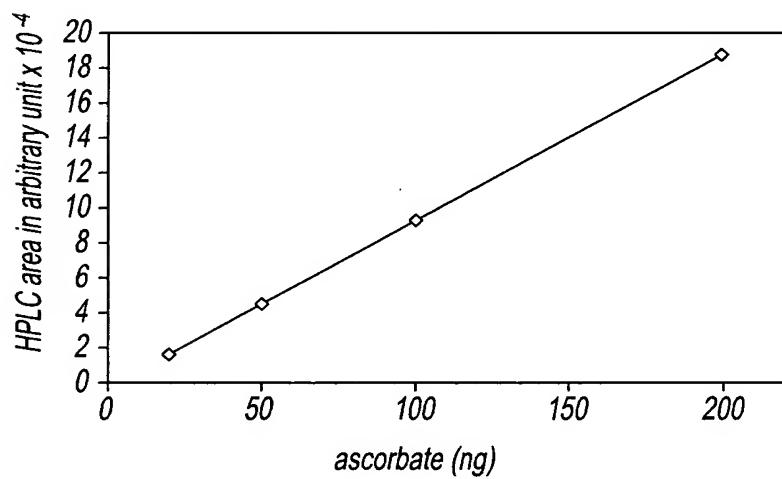
| NO. | PEAK | | VALLEY | |
|-----|----------|------------|----------|------------|
| | nm | Abs | nm | Abs |
| 1 | 293.4 nm | 0.3192 Abs | 250.0 nm | 0.0484 Abs |
| 2 | 223.0 nm | 0.6994 Abs | 215.4 nm | 0.6261 Abs |

Fig. 9

UV spectrophotometric profile of the cs-oxidant in methanol. It has two absorption maxima, one at 293.4 nm and another at 223.0 nm.

*Fig. 10*

Stability of the solid oxidant kept at 25°C under darkness. The stability was determined by its capacity to oxidize ascorbic acid. Ascorbic acid was measured by HPLC analysis at 254 nm.

*Fig. 11*

Standard curve of ascorbic acid based on HPLC analysis at 254 nm.

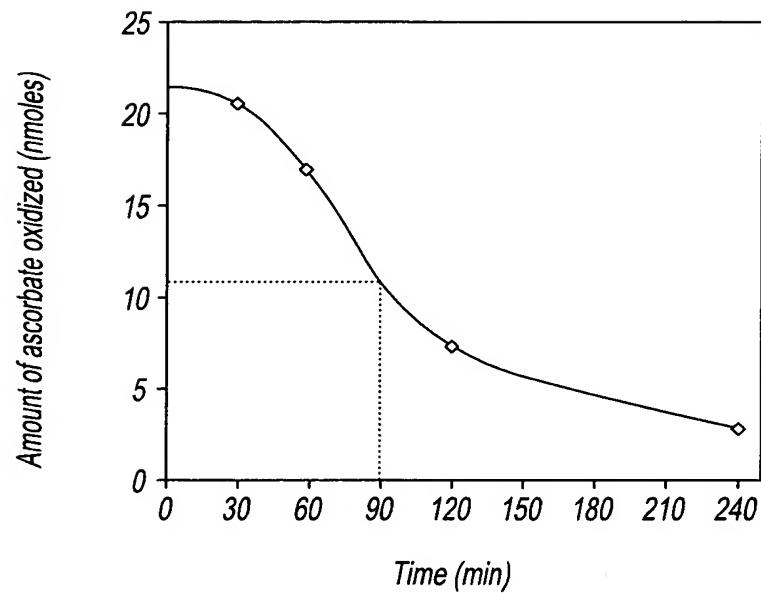


Fig. 12

Stability of the cs-oxidant in 50 mM potassium phosphate buffer at 25°C measured by its potency to oxidize ascorbate as evidenced by HPLC area.

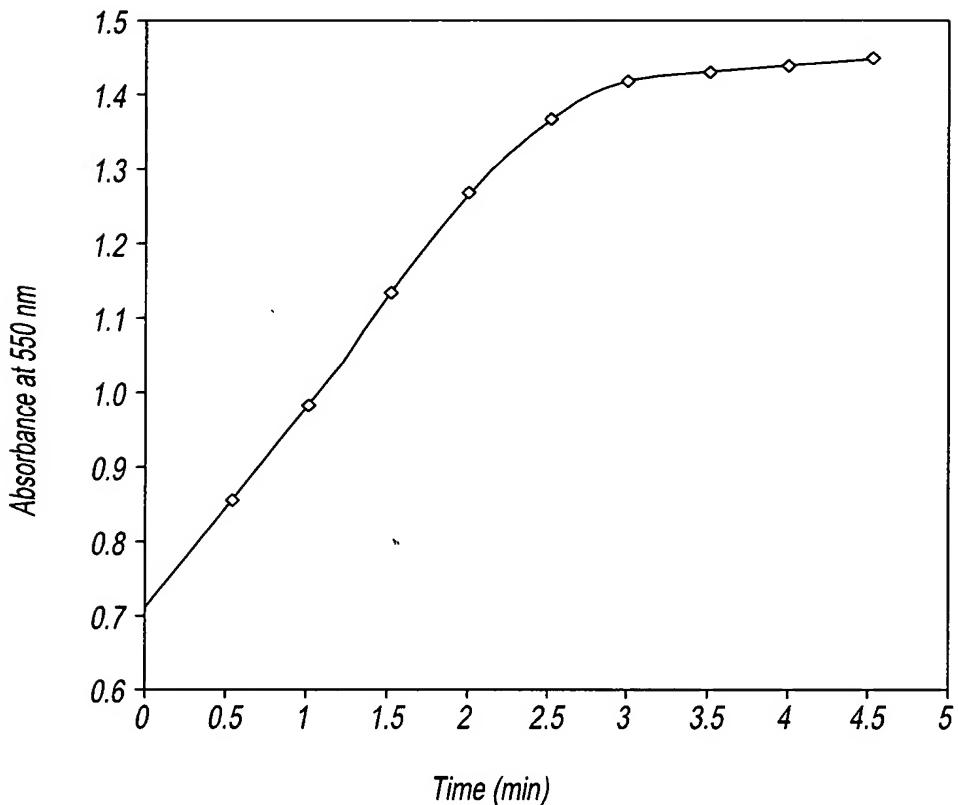


Fig. 13

Quantitative reduction of ferricytochrome c by the oxidant as measured by the formation of ferrocytochrome c with time at 550 nm. The reaction was carried out in 50 mM potassium phosphate buffer, pH 7.4, keeping the final concentration of ferricytochrome c at 100 μ M. One nmole of the oxidant reduced 0.71 nmoles of ferricytochrome c.

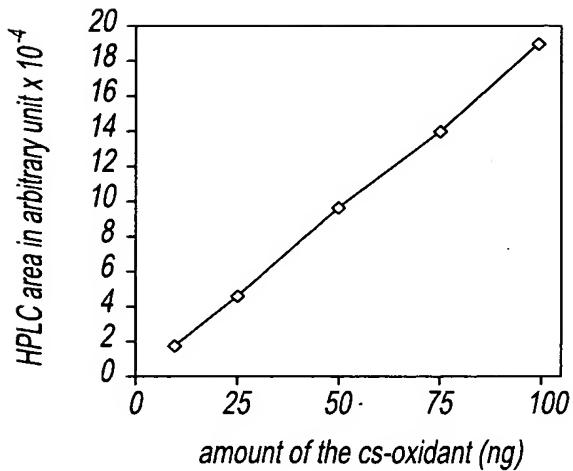


Fig. 14

Standard curve of the oxidant on the basis of HPLC area at 294 nm. Different amounts of the cs-oxidant were used ranging from 10 ng to 100 ng in 20 μ l of mobile solvent.

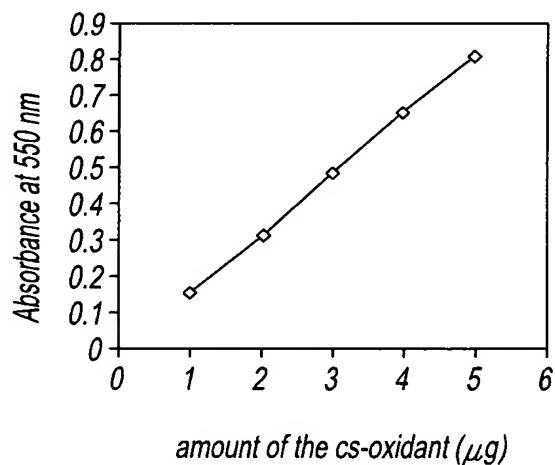


Fig. 15

Standard curve of the oxidant on the basis of reduction of cytochrome c by using different amounts of the oxidant ranging from 1 μ g to 5 μ g.

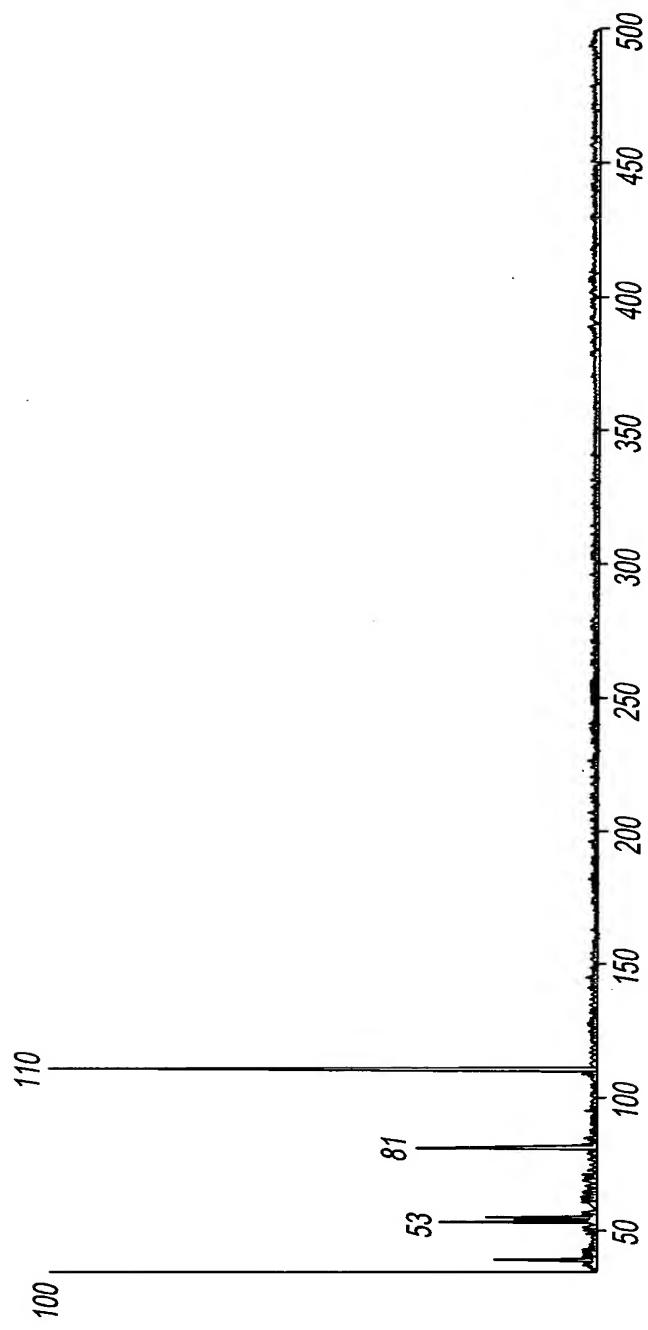
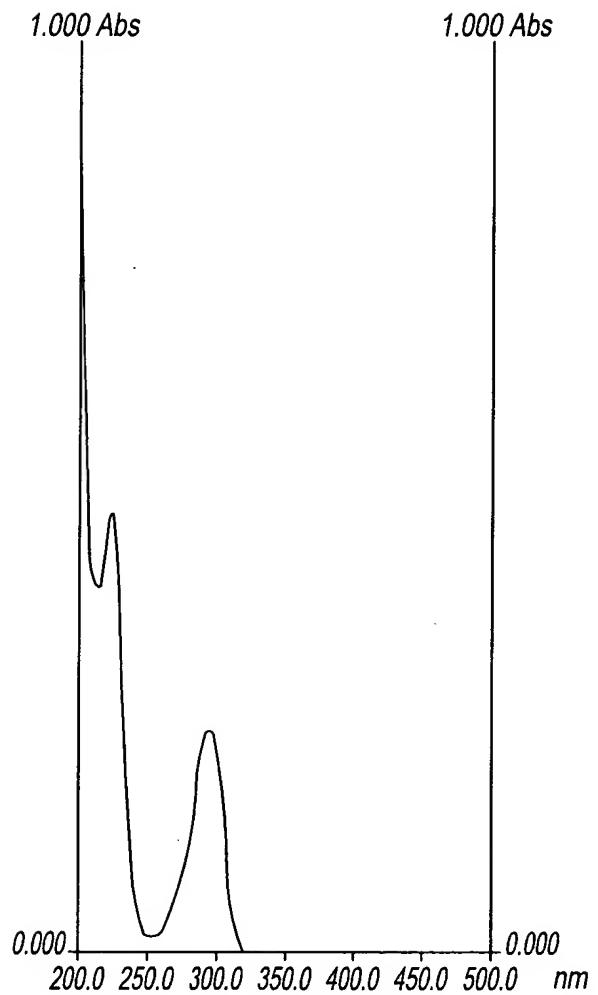


Fig. 16

Mass spectrum of the pure CS-oxidant.



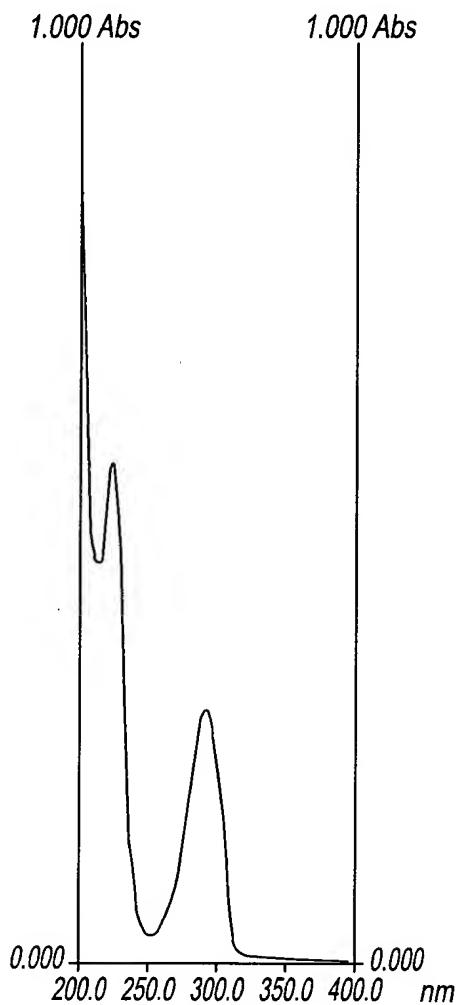
SCAN SPEED: 120.0 nm/min
BANDPASS: 2.00nm

RESPONSE: MEDIUM

| NO. | PEAK | VALLEY | | |
|-----|----------|------------|----------|------------|
| | | | | |
| 1 | 293.8 nm | 0.2443 Abs | 253.0 nm | 0.0137 Abs |
| 2 | 224.2 nm | 0.4837 Abs | 214.4 nm | 0.3979 Abs |

Fig. 17

UV spectrophotometric profile of the hydroquinone in methanol. It has two absorption maxima, one at 293.8 nm and another at 224.2 nm.



SCAN SPEED: 120.0 nm/min

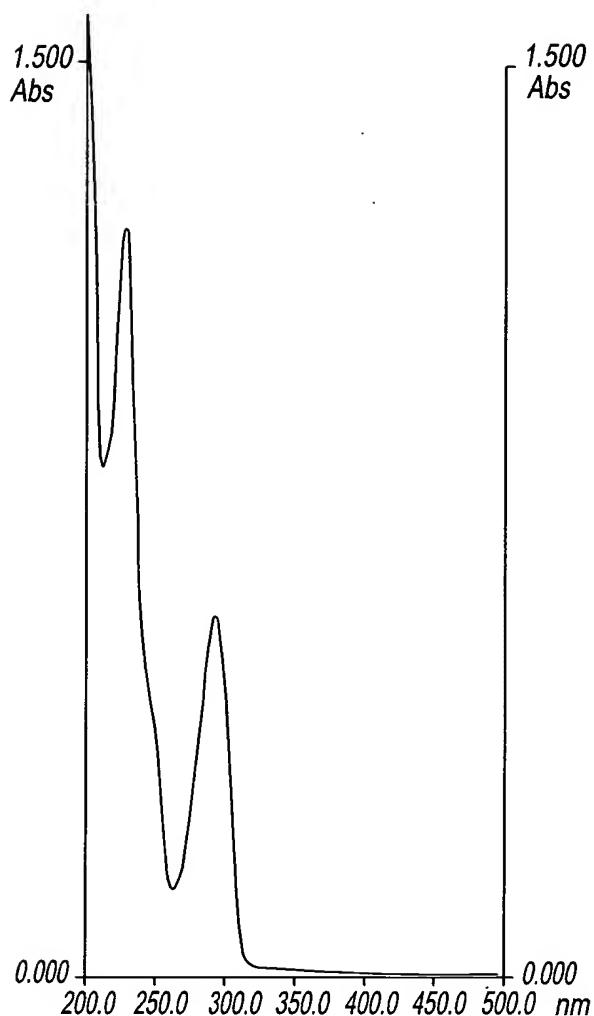
BANDPASS: 2.00nm

RESPONSE: MEDIUM

| NO. | PEAK | | VALLEY | |
|-----|----------|------------|----------|------------|
| | nm | Abs | nm | Abs |
| 1 | 293.6 nm | 0.2772 Abs | 252.8 nm | 0.0269 Abs |
| 2 | 224.4 nm | 0.5476 Abs | 214.0 nm | 0.4314 Abs |

Fig. 18

UV spectrophotometric profile of the cs-oxidant stored at room temperature in dark for 8 days. The two absorption maxima are at 293.6 nm and at 224.4 nm.



SCAN SPEED: 120.0 nm/min

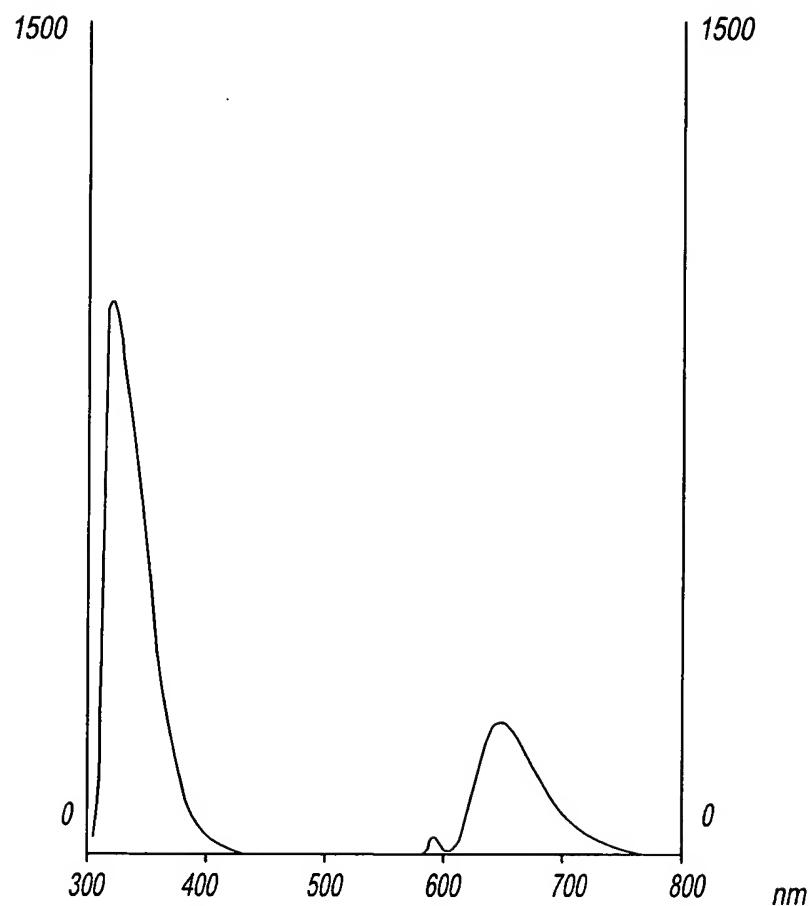
BANDPASS: 2.00nm

RESPONSE: MEDIUM

| NO. | PEAK | | VALLEY | |
|-----|----------|------------|----------|------------|
| 1 | 293.8 nm | 0.5855 Abs | 263.4 nm | 0.1407 Abs |
| 2 | 225.2 nm | 1.2232 Abs | 209.6 nm | 0.8263 Abs |

Fig. 19

UV spectrophotometric profile of equimolar mixture of *p*-benzoquinone and hydroquinone in methanol. There is a shoulder near 242 nm (the λ_{max} of *p*-benzoquinone).



CX WAVELENGTH 294nm

CM BANDPASS 5 nm

CM BANDPASS 5 nm

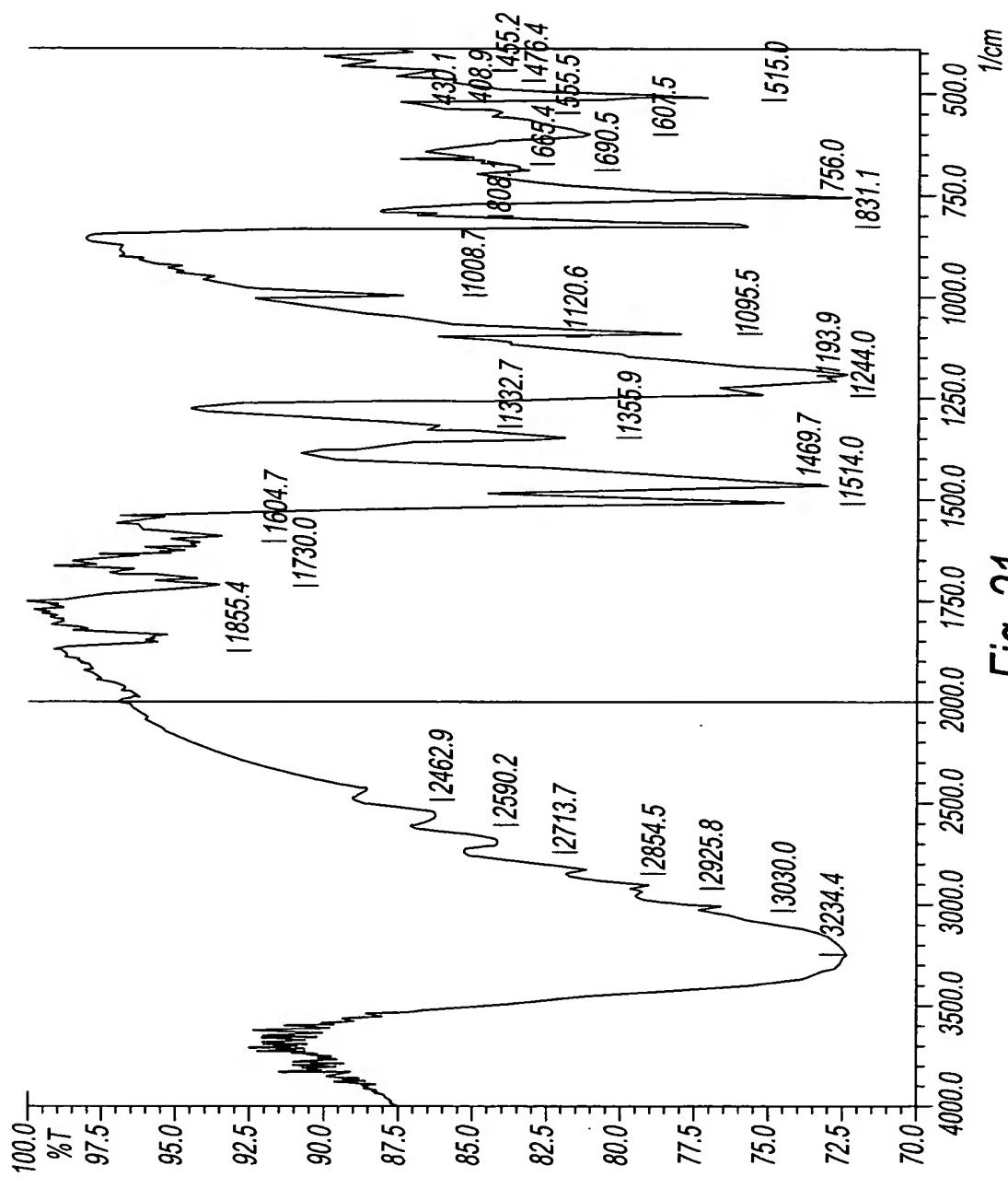
SCAN SPEED: 240 nm/min

RESPONSE 2 sec

| NO. | PEAK | | VALLEY | |
|-----|----------|-----------|----------|-----------|
| | nm | Intensity | nm | Intensity |
| 1 | 329.4 nm | 1000 | 300.2 nm | 20.76 |
| 2 | 593.4 nm | 35.50 | 564.6 nm | 0.477 |
| 3 | 651.6 nm | 243.5 | 684.2 nm | 7.546 |

Fig. 20

Fluorescence spectroscopic profile of the hydroquinone in methanol. The excitation was at 294 nm and the emission scanning was measured from 300 nm to 800 nm. The emission maxima were at 329.4 nm and at 651.6 nm.

**Fig. 21**

FTIR spectroscopic profile of the cs-oxidant.

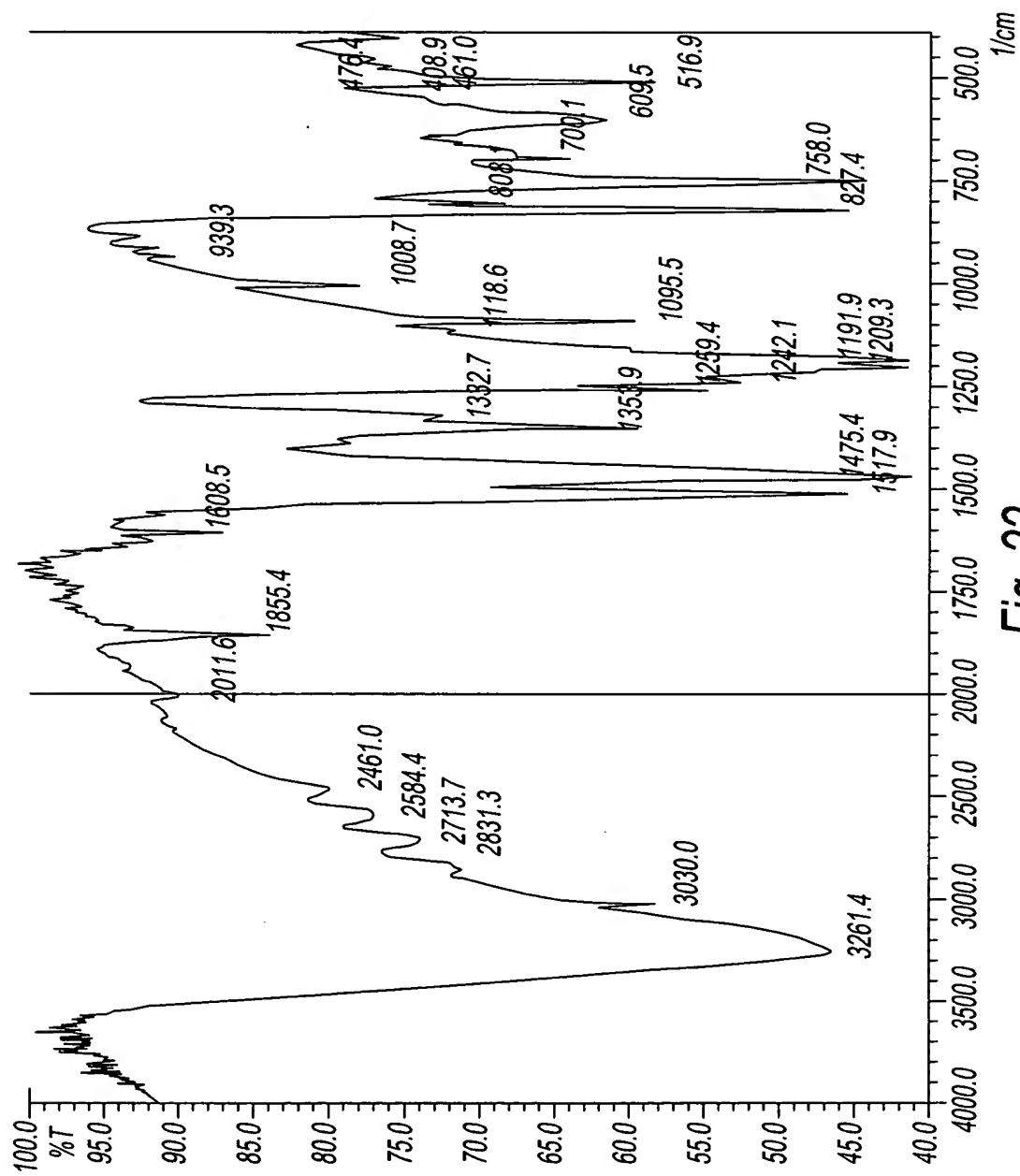


Fig. 22

FTIR Spectroscopic profile of hydroquinone.

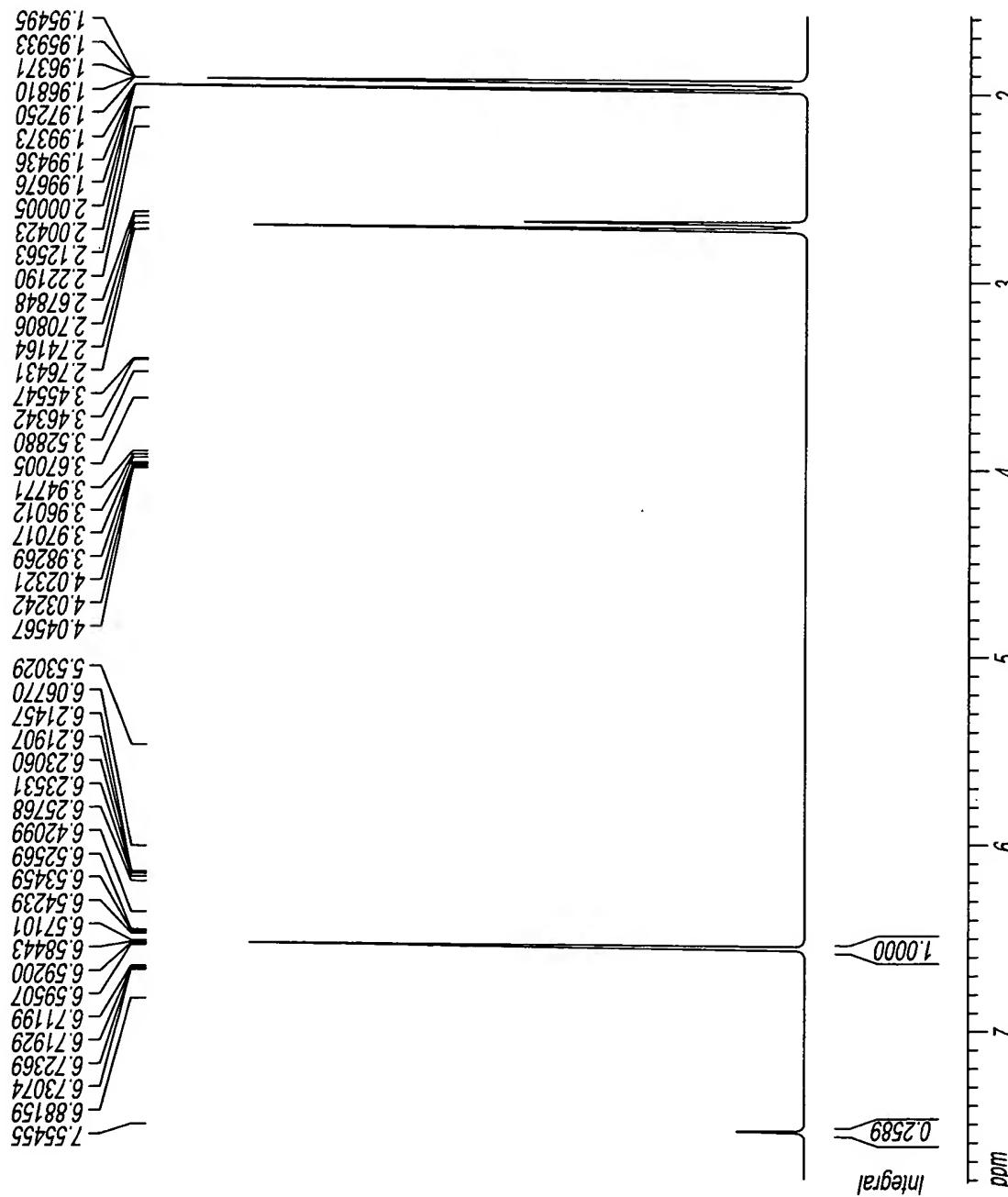


Fig. 23

$^1\text{H-NMR}$ spectroscopic profile of the co-oxidant in CD_3COCD_3 .

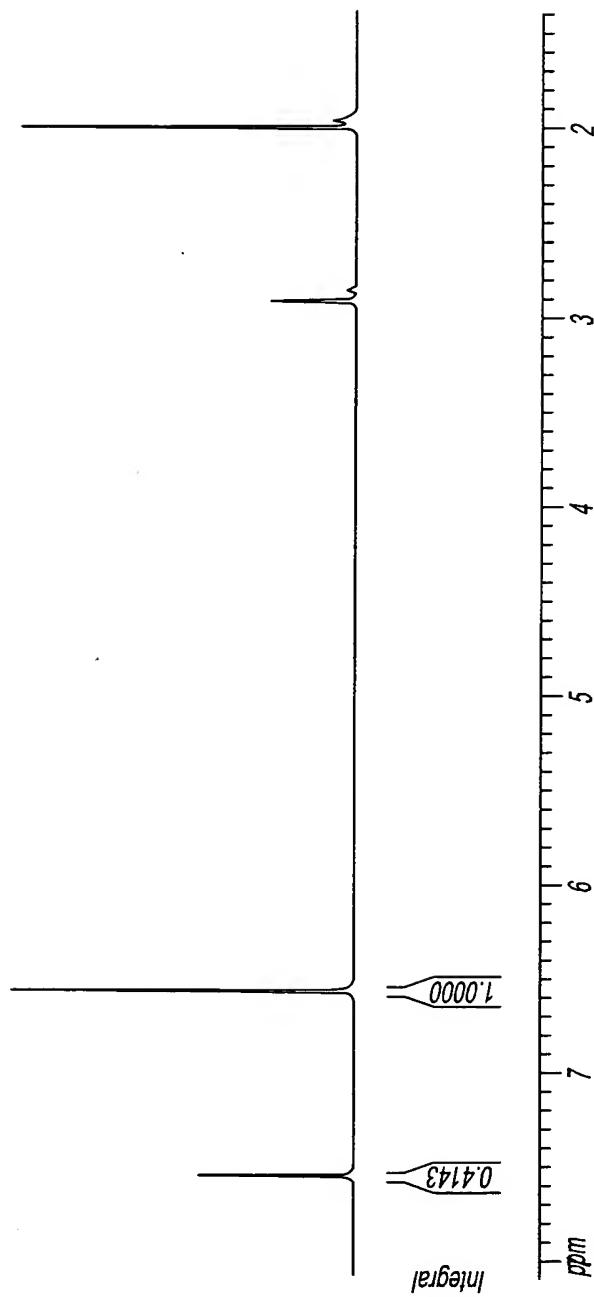


Fig. 24

$^1\text{H-NMR}$ spectroscopic profile of hydroquinone in CD_3COCD_3 .

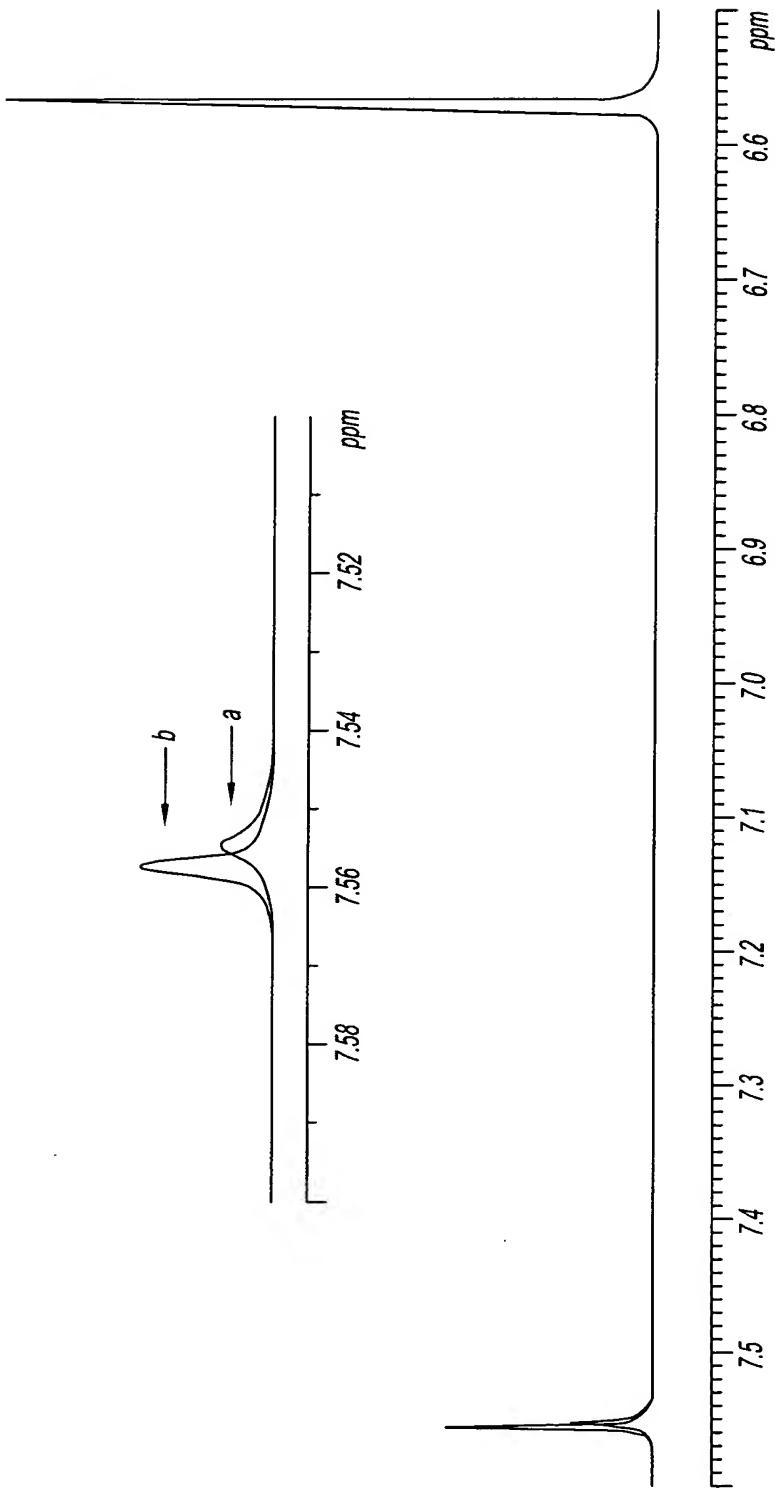


Fig. 25

Comparative ¹H-NMR spectroscopic profiles of (a) cs-oxidant and (b) hydroquinone.

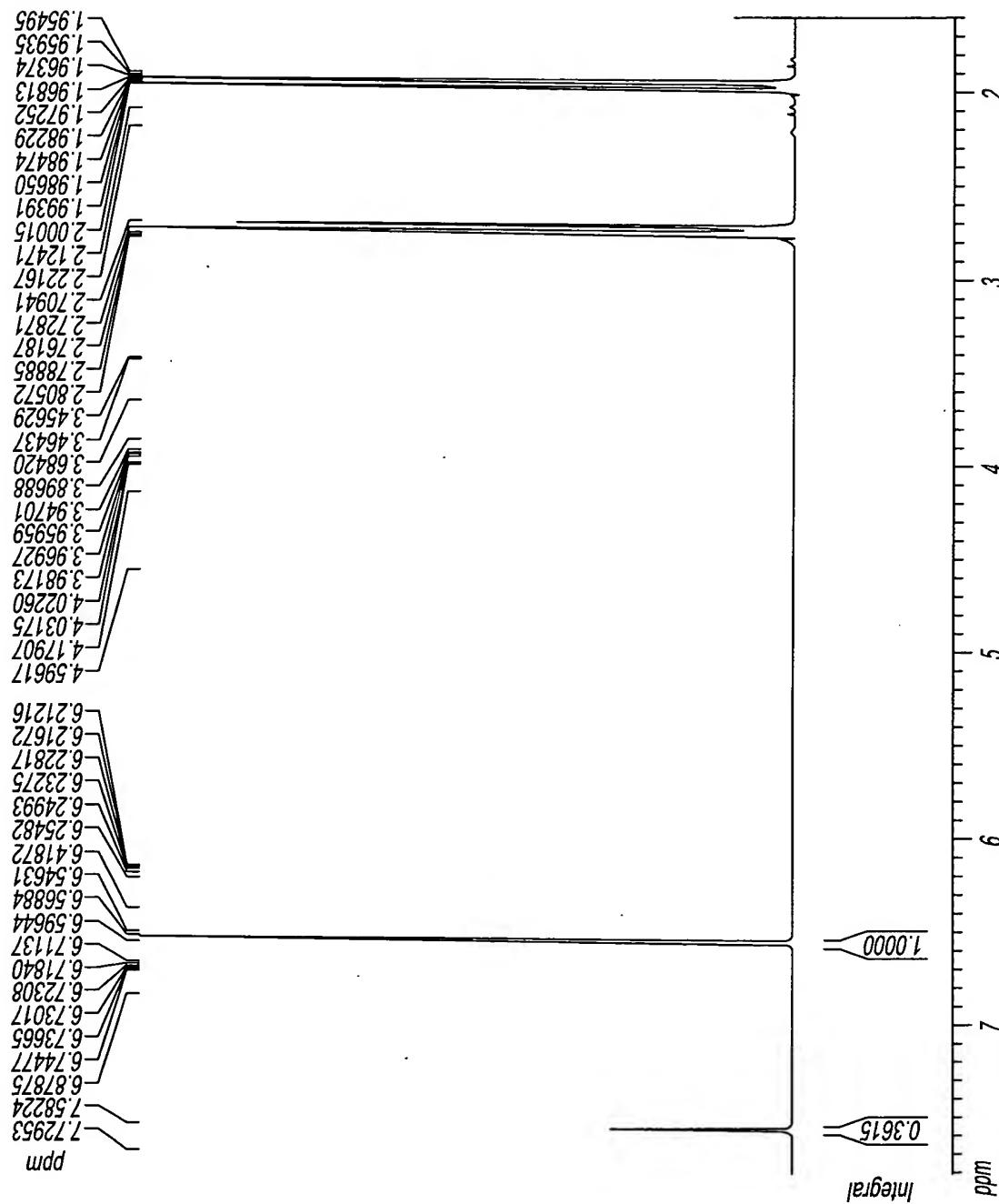


Fig. 26
 $^1\text{H-NMR}$ spectroscopic profile of the co-oxidant after reduction with sodium dithionite.

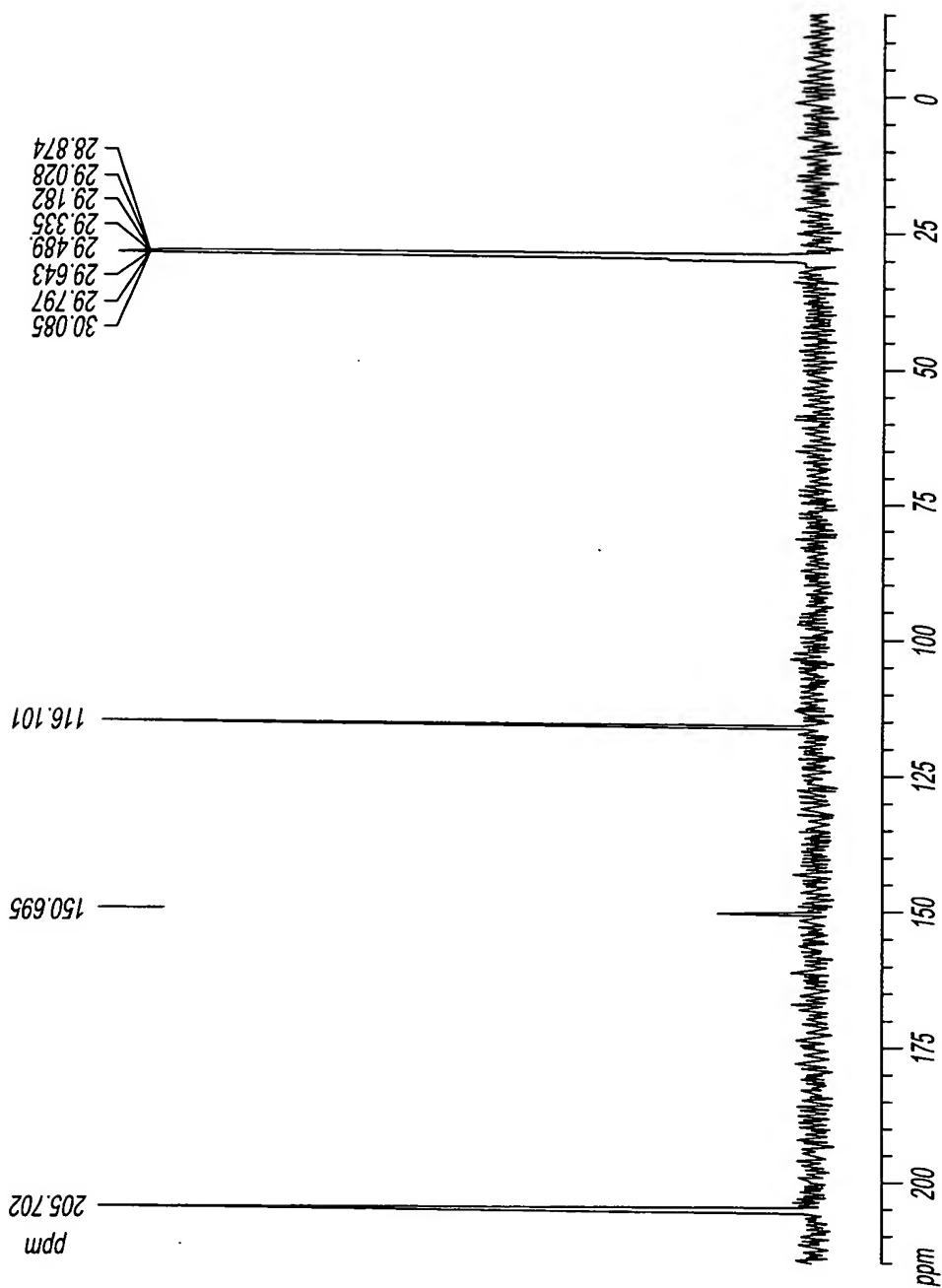


Fig. 27

^{13}C -NMR spectroscopic profile of the cs-oxidant in CD_3COCD_3 .

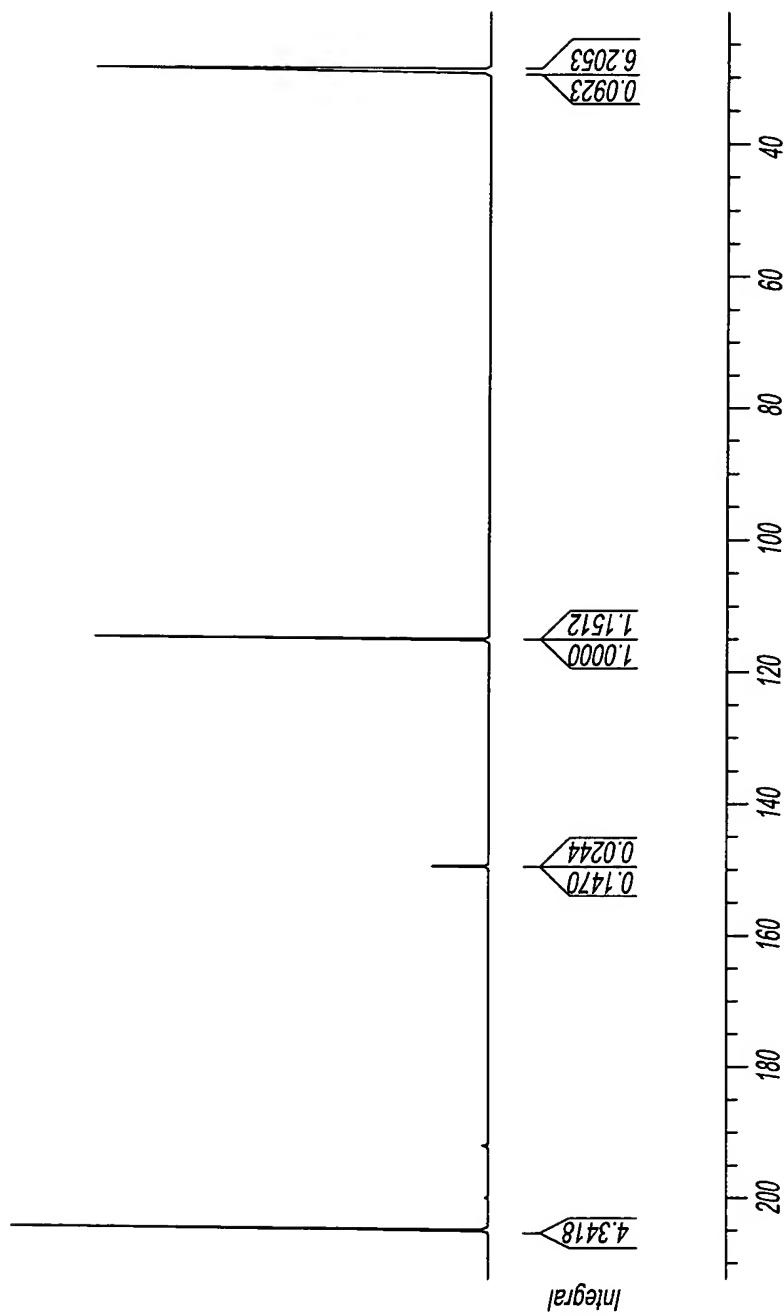


Fig. 28

C-NMR spectroscopic profile of hydroquinone in CD_3COCD_3 .

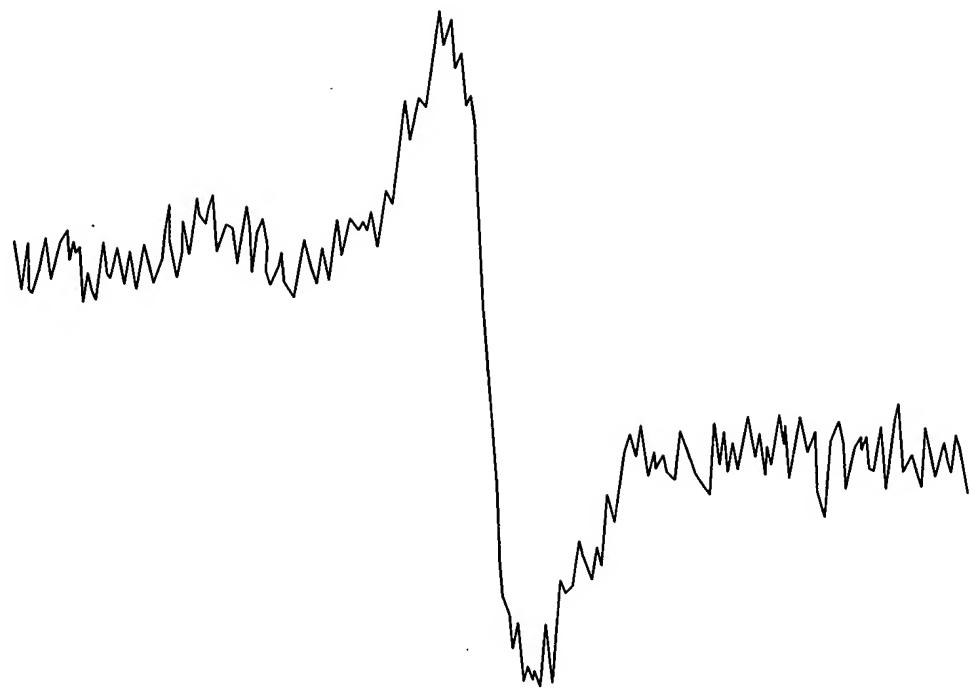


Fig. 29

Room temperature ESR spectrum of cs-oxidant, freshly prepared from 100 cigarettes. The spectrum was recorded on a JES-REIX ESR spectrometer (Tokyo, Japan). The spectral parameters were as follows: microwave frequency, 9.435 GHz; power, 2mW; field modulation width, 0.4mT; modulated frequency, 100 kHz; time constant, 0.3 sec; scan rate, 2.5 mT/sec.

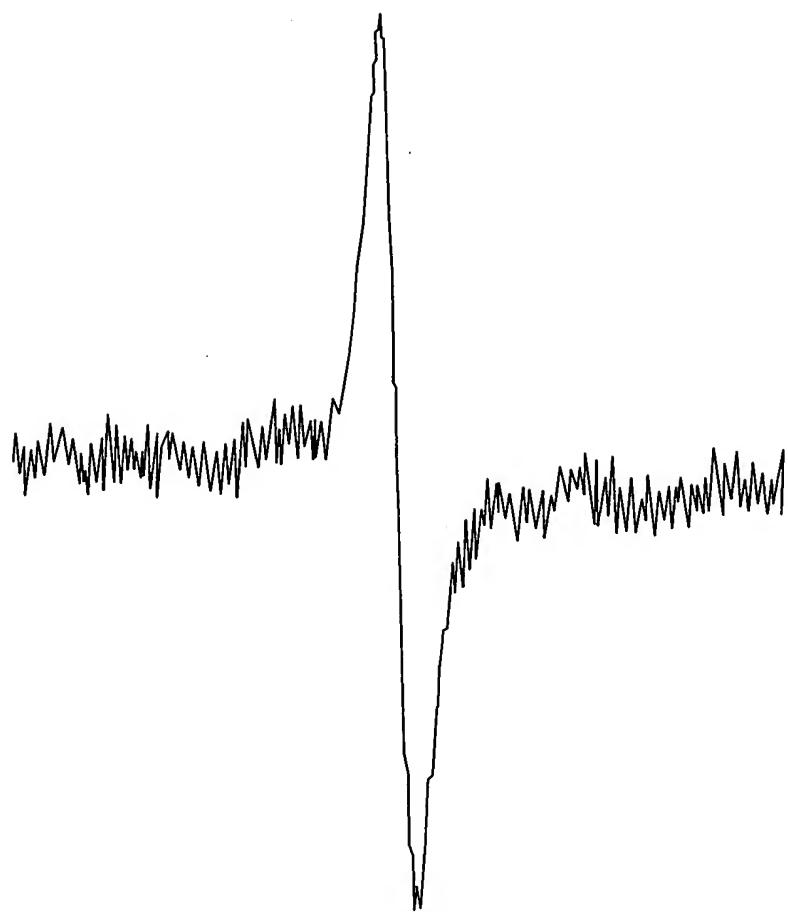
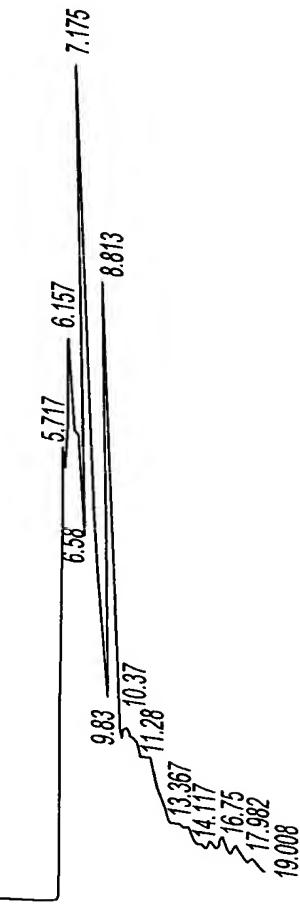


Fig. 30

Room temperature ESR spectrum of aged (10 days) cs-oxidant, prepared from 400 cigarettes.



| CHROMATOPAC | | C-R6A | FILE | 0 |
|-------------|--------|---------|------|-----------|
| SAMPLE NO | 0 | METHOD | 41 | |
| REPORT NO | 48 | | | |
| PKNO | TIME | AREA | MK | IDNO |
| 1 | 5.717 | 475376 | | 19.0777 |
| 2 | 6.157 | 317530 | V | 12.7431 |
| 3 | 6.58 | 209664 | V | 8.4142 |
| 4 | 7.175 | 708579 | V | 28.4366 |
| 5 | 8.813 | 340583 | V | 13.6682 * |
| 6 | 9.83 | 99028 | V | 3.9742 |
| 7 | 10.37 | 103590 | V | 4.1573 |
| 8 | 11.28 | 178509 | V | 7.1639 |
| 9 | 13.367 | 24236 | V | 0.9727 |
| 10 | 14.117 | 15200 | V | 0.61 |
| 11 | 16.75 | 9187 | | 0.3687 |
| 12 | 17.782 | 10303 | | 0.4135 |
| TOTAL | | 2491784 | | 100 |

Fig. 31

HPLC profile of the whole cs solution analyzed in the silica column (LiChrospher® Si 60, Merck).
 * indicates the retention time, area and the concentration (13.6682%) of the cs-oxidant.

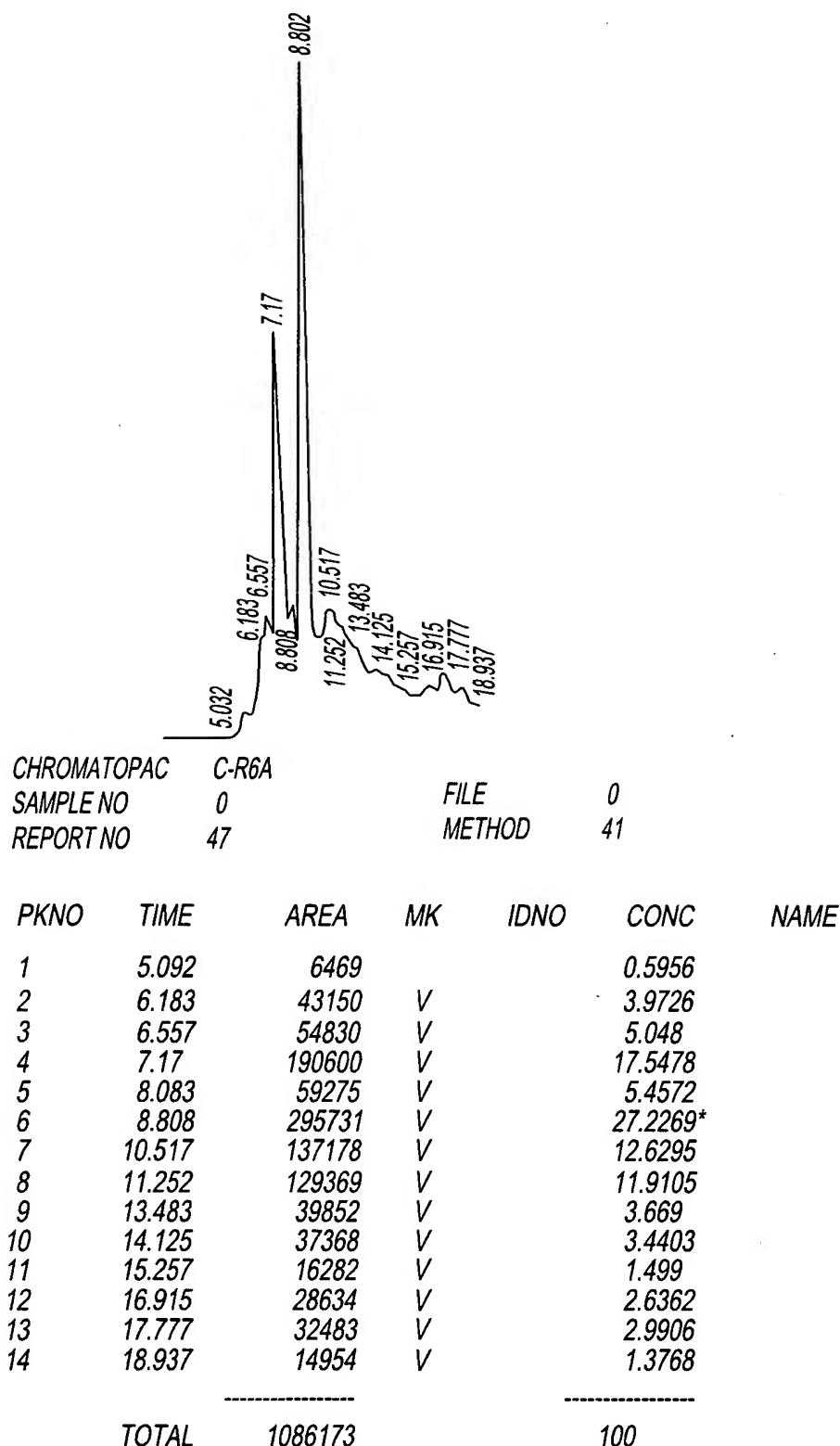


Fig. 32

HPLC profile of the aqueous extract of cs solution analyzed in the silica column (LiChrospher® Si 60, Merck).

* indicates the retention time, area and the concentration (27.2269%) of the cs-oxidant.

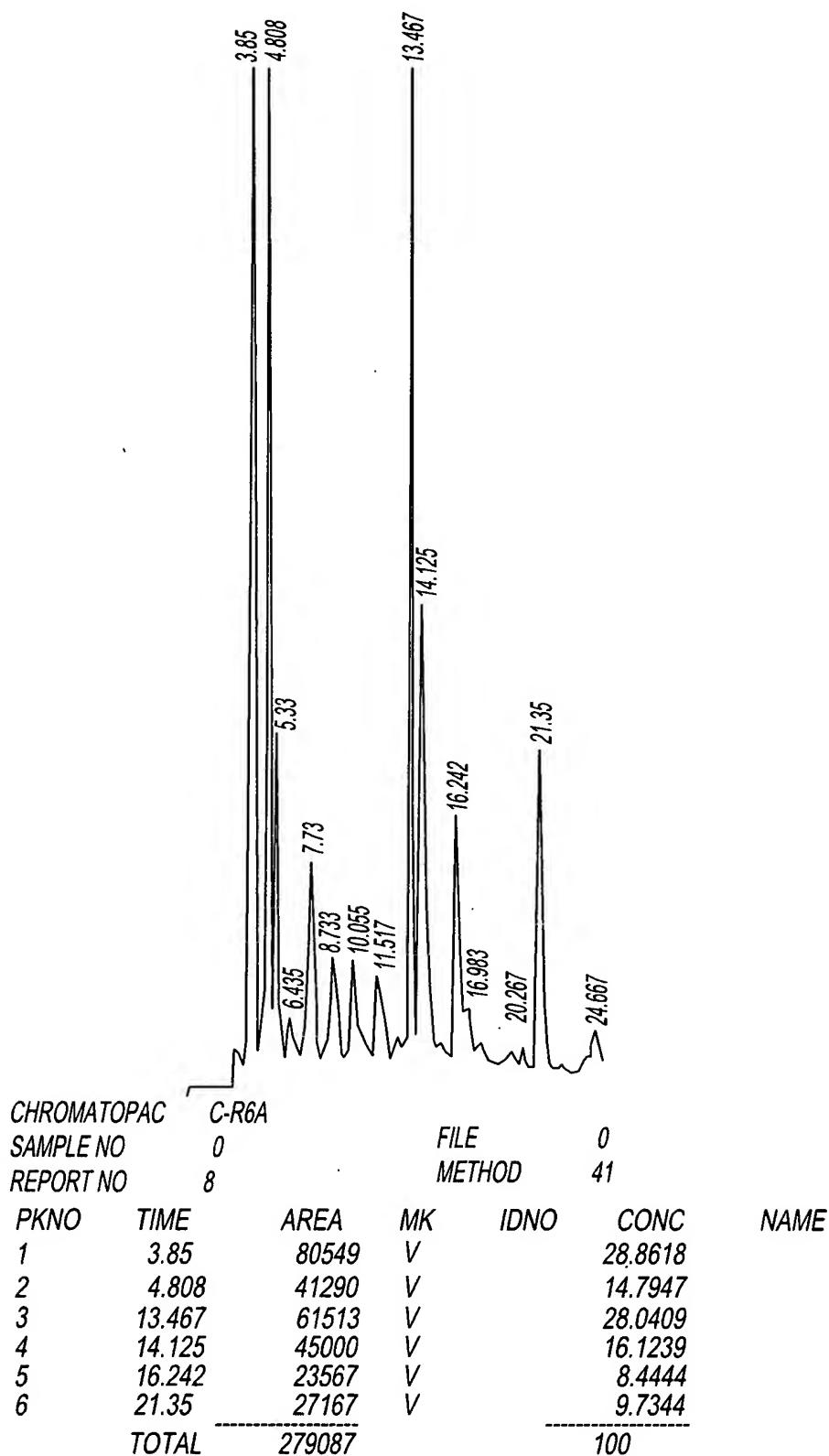
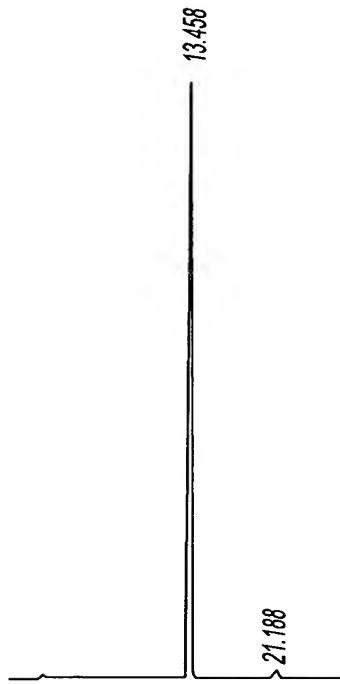


Fig. 33

HPLC profile of the whole cs solution analyzed in the ODS column (Shim-pack CLC -ODS, Shimadzu).
The cs-oxidant eluted at 13.467 min.



| | | | | |
|-------------|-------|--------|----|--|
| CHROMATOPAC | C-R6A | | | |
| SAMPLE NO | 0 | FILE | 0 | |
| REPORT NO | 9 | METHOD | 41 | |

| PKNO | TIME | AREA | MK | IDNO | CONC | NAME |
|-------|--------|--------|-------|------|------|------|
| 1 | 13.458 | 162863 | | | 100 | |
| <hr/> | | | <hr/> | | | |
| | TOTAL | 162863 | | | 100 | |

Fig. 34

HPLC profile of the pure cs-oxidant, analyzed in the CLC-ODS column (Shim-pack CLC-ODS, Shimadzu) eluted at the retention time of 13.458 min.

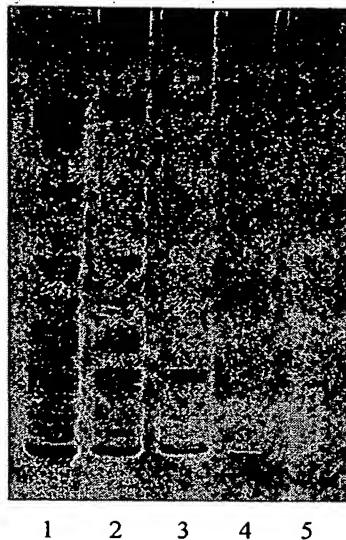


Fig. 35a

SDS-PAGE of the guinea pig lung microsomal proteins treated with whole cs solution and the cs-oxidant. Lane 1, untreated microsomes; lane 2, microsomes treated with 50 μ l cs solution; lane 3, microsomes treated with 100 μ l cs solution; lane 4, microsomes treated with 10 μ g cs-oxidant; lane 5, microsomes treated with 20 μ g cs-oxidant.

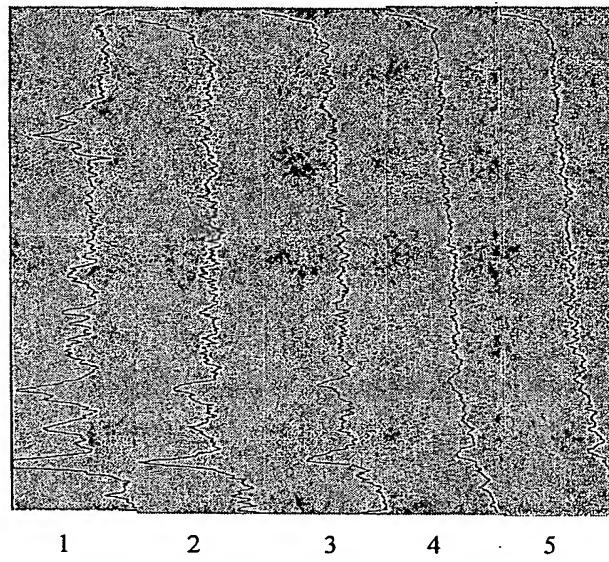


Fig. 35b

Densitometric scanning of the protein bands of different lanes as in Fig. 35a.